PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12M 3/00, C12N 5/00

A1

(11) International Publication Number: **WO 99/33951**

(43) International Publication Date: 8 July 1999 (08.07.99)

(21) International Application Number: PCT/US98/27845

(22) International Filing Date: 30 December 1998 (30.12.98)

(30) Priority Data:
09/001,609 31 December 1997 (31.12.97) US

(71) Applicant: ADVANCED TISSUE SCIENCES, INC. [US/US]; 10933 North Torrey Pines Road, La Jolla, CA 92037-1005 (US).

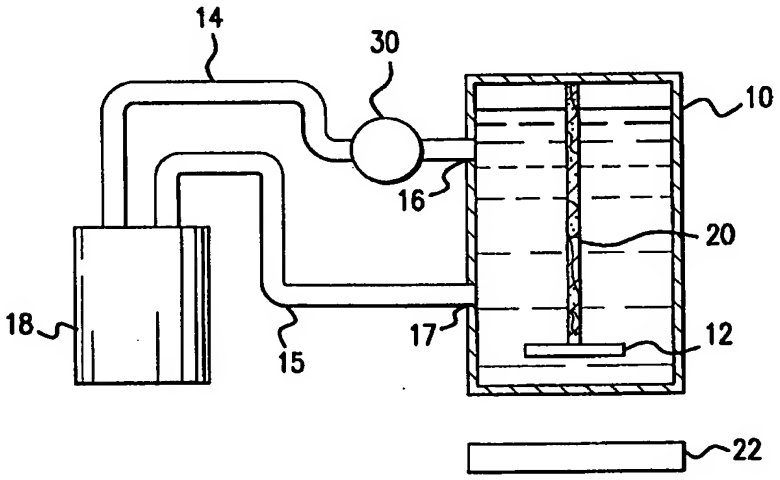
(72) Inventors: PETERSON, Alvin; 17620 Skyline Truck Terrace, Jamul, CA 91935 (US). LANDEEN, Lee, K.; 3677 First Avenue, San Diego, CA 92103 (US). BENNETT, John; 3604 Arnold Avenue, San Diego, CA 92104 (US). GEE, Jason; 12658 Lupita Court, San Diego, CA 92130 (US). CHESLA, Scott; 500 Northside Circle Northwest, Atlanta, GA 30309 (US). ZELTINGER, Joan; 4136 Camino Ticino, San Diego, CA 92122 (US). FLATT, James, H.; 14121 Half Moon Bay Drive, Del Mar, CA 92014 (US). APPLEGATE, Mark, A.; 4468 Saratoga Avenue, San Diego, CA 92107 (US). DUNKELMAN, Noushin; 11518 Mesa Madera Court, San Diego, CA 92131 (US). KEMMERRER, Stephen, V.; 12595 Ragweed Street, San Diego, CA 92129 (US).

(74) Agents: CORUZZI, Laura, A. et al.; Pennie & Edmonds LLP, 1155 Avenue of the Americas, New York, NY 10036 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: APPARATUS AND METHOD FOR SIMULATING IN VIVO CONDITIONS WHILE SEEDING AND CULTURING THREE-DIMENSIONAL TISSUE CONSTRUCTS



(57) Abstract

An apparatus and method for sterilizing, seeding, culturing, storing, shipping, and testing three-dimensional tissue constructs is disclosed. The apparatus includes a fluid reservoir (18), a pump (30), at least one treatment chamber (10), and a means for controlling media flow characteristics around a tissue construct (20) disposed within the treatment chamber (10), and for controlling movement of the construct itself, so as to simulate a variety of physiologic conditions. One exemplary embodiment of the invention includes a means for applying an axial stress to the construct. Applying an axial stress to the construct during seeding and culturing results in a tissue-engineered construct with cells and their fibers oriented in a manner which is more likely to possess long term dimensional stability and the patency of native vessels with normal physiologic function.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

APPARATUS AND METHOD FOR SIMULATING IN VIVO CONDITIONS WHILE SEEDING AND CULTURING THREE-DIMENSIONAL TISSUE CONSTRUCTS

Alvin Peterson
Lee K. Landeen
John Bennett
Jason Gee
Scott Chesla
Joan Zeltinger
James Flatt
Mark A. Applegate
Noushin Dunkelman
Stephen Kemmerrer

RELATED APPLICATIONS

This application is a continuation-in-part of United States patent application serial number 08/478,309, filed June 7, 1995 and entitled "Apparatus and Method for Sterilizing, Seeding, Culturing, Storing, Shipping, and Testing Tissue, Synthetic, or Mechanical Heart Valves or Valve Segments;" United States patent application serial number 08/912,948, filed August 14, 1997 and entitled "Apparatus and Method for Sterilizing, Seeding, Culturing, Storing, Shipping, and Testing Replacement Cartilage Tissue Constructs," which is a continuation of United States patent application serial number 08/486,185, filed June 7, 1995 and entitled "Apparatus and Method for Sterilizing, Seeding, Culturing, Storing, Shipping, and Testing Replacement Cartilage Tissue Constructs;" and United States patent application serial number 08/672,697, filed June 27, 1996 and entitled "Apparatus and Method for Sterilizing, Seeding, Culturing, Storing, Shipping, and Testing Tissue, Synthetic, or Native Vascular Grafts," which is a continuation-in-part of United States patent application serial number 08/430,768, filed April 27, 1995 and entitled "Apparatus and Method for Sterilizing, Seeding, Culturing, Storing, Shipping, and Testing Tissue, Synthetic, or Native Vascular Grafts."

Each of the above-referenced applications is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Technical Field

The present invention relates to the sterilization, seeding, culturing, storing, shipping, and testing of three-dimensional tissue. Specifically, the present invention relates to an apparatus and method for seeding and culturing three-dimensional tissue constructs with viable mammalian cells under simulated in vivo conditions, resulting in three-dimensional tissue that is more likely to display the biochemical, physical, and structural properties of native tissues.

Discussion of the Related Art

Biological implants are presently used by surgeons to repair or replace a variety of native tissues, including heart valves, arterial or venous blood vessels, articular cartilage, tendons, and ligaments, that are weakened, damaged or obstructed due to trauma or disease. Historically, implants have been either homografts, prosthetic grafts made of synthetic materials such as polyester (e.g., Dacron), expanded polytetraflouroethylene (ePTFE), and other composite materials, or fresh or fixed biological tissue grafts.

However, synthetic grafts generally have inadequate patency rates for many uses, while the harvesting of homografts requires extensive surgery which is time-consuming, costly, and traumatic to the patient. Fixed tissue grafts do not allow for infiltration and colonization by the host cells, which is essential to remodeling and tissue maintenance. Consequently, fixed tissue grafts degrade with time and will eventually malfunction.

Due to the inadequacies of these currently available synthetic and biological grafts, as well as the cost and limited supply of homografts, tissue-engineered grafts are being developed which are seeded and cultured, in vitro, with

cells. For example, U.S. Patent No. 5,266,480 to Naughton et al. discloses the establishment of a three-dimensional matrix, seeding of the matrix with desired cells, and maintenance of the culture to provide a variety of three-dimensional tissues suitable for use in different applications. Tissue-engineered grafts utilizing this technology may be superior to other grafts for use in replacement therapy in that they more closely display the long term dimensional stability and patency of native arteries and vessels with normal physiologic functionality.

Historically, the seeding and culturing of such grafts, and tissue in general, has taken place in a static environment such as a Petri or culture dish. However, there are disadvantages to seeding and culturing tissue in such an environment. For example, the lack of circulation of nutrients in these static systems results in a slow and ineffective seeding and culturing process. Moreover, a static culturing environment may lead to de-differentiation and loss of tissue function, and cannot support growth of tissue beyond a certain thickness.

In contrast, tissues that are seeded and cultured in a dynamic environment can be grown to a wider range of thicknesses, and are more likely to tolerate the physiological conditions that exist in the human body once implanted. Thus, there exists a need for an environment that is designed to simulate physiologic conditions that particular tissues would be subjected to in vivo, in which to seed and culture tissue-engineered grafts and other prosthetic devices.

SUMMARY OF THE INVENTION

It is therefore an object of the invention to provide an apparatus for seeding and culturing tissue constructs which is designed to simulate physiologic conditions that a

particular construct would be subjected to in vivo by controlling the growth media flow through and across the construct, and the movement of the construct itself, to create varying physiologic-like pressures and forces which act upon the growing tissue.

It is a further object of the invention to stimulate the production of replacement tendon and ligament tissue constructs which display the critical biochemical, physical, and structural properties of native human tendon and ligament tissue by seeding and culturing the tissue in a dynamic environment.

It is a further object of the invention to provide a precise mechanical device with a minimum of moving parts to provide such environments.

It is yet a further object of the invention to provide a closed system free from contamination for sterilizing, seeding, culturing, storing, shipping, and testing tissue constructs.

In accordance with the present invention, there is provided an apparatus and method for seeding and culturing tissue constructs with viable mammalian cells under simulated in vivo conditions, resulting in three-dimensional tissue that is more likely to display the biochemical, physical, and structural properties of native tissues.

One exemplary embodiment of an apparatus according to the invention comprises a fluid reservoir, at least one construct treatment chamber, a support structure for supporting the construct in the treatment chamber, and a means for placing an axial load on the construct. By placing an axial load on the construct in the treatment chamber during culturing, an axial stress is placed on the construct. This stress results in a tissue-engineered tendon or ligament construct with cells and their fibers oriented in a manner which is more likely to possess long term dimensional

stability and the patency of, for example, native tendons or ligaments with normal physiologic function. In this manner, one embodiment of the invention advantageously utilizes a mechanically non-complex apparatus to create a dynamic environment in which to seed and culture tissue-engineered tendons, ligaments or other implantable devices.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become more readily apparent from the following detailed description, which should be read in conjunction with the accompanying drawings in which:

FIG. 1 illustrates an apparatus according to the present invention for sterilizing, seeding, culturing, storing, shipping, and testing a prosthesis in which a magnetic axial loading is utilized;

FIG. 2 illustrates an apparatus according to the present invention for sterilizing, seeding, culturing, storing, shipping, and testing a prosthesis in which a mechanical axial loading is utilized, and wherein the mechanical loading is generated using a piston;

FIG. 3 illustrates an apparatus according to the present invention for sterilizing, seeding, culturing, storing, shipping, and testing a prosthesis in which a mechanical axial loading is utilized, and wherein the mechanical loading is generated using a bellows; and

FIG. 4 illustrates yet another alternative exemplary embodiment of an apparatus according to the present invention for sterilizing, seeding, culturing, storing, shipping, and testing a prosthesis in which a flexible diaphragm is utilized to provide an axial load.

DETAILED DESCRIPTION OF THE INVENTION

The following embodiments of the present invention will be described in the context of an apparatus and method for sterilizing, seeding, culturing, storing, shipping, and testing tendon and ligament constructs, although those skilled in the art will recognize that the disclosed methods and structures are readily adaptable for broader application. Note that whenever the same reference numeral is repeated

with respect to different figures, it refers to the corresponding structure in each such figure.

FIG. 1 discloses a system for sterilizing, seeding, culturing, storing, shipping, and testing tendon and ligament constructs. According to a preferred embodiment of the invention, this system primarily comprises a treatment chamber 10, a pump 30, and a media reservoir 18 fluidly communicating with the treatment chamber.

Media reservoir 18 is used to store fluid for the system. Illustrative suitable reservoirs are the Gibco-BRL 1L media bag or any rigid container capable of sterilization. Reservoir 18 may include a one way sterile filter so as to provide a direct source of filtered gas to the fluid within the system or, alternatively, may include gas-permeable tubing or membranes comprised of a material such as silicone or Teflon so as to provide an indirect source of sterile gas to the system via diffusion. Examples of fluid which may be used in the system include, but are not limited to, sterilizing fluid, tanning fluid, cryopreservative fluid, rinse fluid, fluid containing cells, or fluid containing a culture medium. It is to be understood that during testing, seeding, and culturing in a preferred embodiment, the fluid is advantageously kept at human body temperature through use of an incubator, for example, and may be composed of a fluid which approximates the viscosity of human blood or other human bodily fluids.

The fluid contained in reservoir 18 is retrieved through either fluid line 14 or 15 into treatment chamber 10 through the action of pump 30, which, as discussed below, controls the flow of fluid within the system. Fluid lines 14 and 15, as well as all other fluid lines in the system, may be made of any type of stainless steel tubing or medical grade, sterilizable, durable, plastic tubing preferably comprised of

a gas-permeable material such as silicone which is suitable for transporting the fluid in use.

Treatment chamber 10 is preferably composed of any biocompatible, rigid material capable of being sterilized such as Teflon, polycarbonate, PVC, stainless steel, acrylic, polypropelene, and polyethylene. Treatment chamber 10 may be a single piece of material or may comprise two or more sections which are secured and made leak proof through any standard means such as inner and outer threads, an o-ring seated in an annular groove, a clamp, or bonding agents. In order to view construct 20 within treatment chamber 10, a viewing port may be placed at any point on the chamber, or alternatively, the chamber may be made of an optically clear material such as polycarbonate or PVC. Ports 16 and 17 of treatment chamber 10 allow for the perfusion and/or circulation of fluid into and through chamber 10. Ports 16 and 17 are also used to attach treatment chamber 10 to fluid lines 14 and 15 respectively.

As mentioned, pump 30 controls the flow of the fluid within treatment chamber 10. Pump 30 may be any pump capable of providing unidirectional or bidirectional pulsatile or continuous pressure fluid flow in the system. Examples of such pumps include but are not limited to low flow pumps, invasive pumps such as a lobe-type pump, or non-invasive pumps such as a peristaltic pump. Thus, illustratively, when pump 30 is operating so as to force fluid from reservoir 18 to fluid line 14, and subsequently from fluid line 14 into chamber 10, fluid is forced from port 16 through chamber 10 to port 17. However, if pump 30 is operated so as to provide fluid flow in the opposite direction, fluid is then forced in an opposite direction from port 17 through chamber 10 to port 16.

It is to be understood that the pressure from pump 30 may be varied during use so as to provide varying pressure

within treatment chamber 10. Moreover, it is to be understood that pump 30 may be operated bidirectionally in any manner and at any interval. Thus, pump 30 may be operated so as to provide bidirectional fluid flow in an alternating fashion at some predetermined interval. Alternatively, pump 30 may provide fluid flow in one direction for a predetermined period of time followed by fluid flow in the opposite direction for a similar period of time, or may provide fluid flow solely in one direction. Moreover, it is to be understood that, alternatively, one skilled in the art could devise a suitable valving system (e.g., through the use of a rotating valve) that could also provide chamber 10 with a bidirectional fluid flow.

Treatment chamber 10 is configured and dimensioned to house a tendon or ligament construct 20. Construct 20 may illustratively consist of any knitted, braided, woven, felted, or synthesized material that is bioresorbable and/or biocompatible, as well as any native material which will support appropriate cells. Treatment chamber 10 may be made any size so as to hold a construct 20 of any length or diameter. This is advantageous, as the system may be used to sterilize, seed, culture, store, ship, and test constructs of any size.

In a preferred embodiment of the present invention, construct 20 is secured to the top of treatment chamber 10 by any well known means. Such means include, but certainly are not limited to, sewing, lashing, adhesive bonding, clamping, welding, staking, pinching, or heat bonding.

A magnet 12 is attached to the lower end of construct 20 by any similarly well known means, such as those listed above. Magnet 12 may be comprised of any material that has the property of producing a magnetic field external to itself, including iron, steel or an alloy that has had this property artificially imparted. Alternatively, magnet 12 may

be any material that has the property of being attracted by an external magnetic field.

As shown in FIG. 1, also included within the system is a magnetic field generator 22 for applying a magnetic field to magnet 12. Magnetic field generator 22 includes a device or material that is capable of producing a magnetic field, such as a magnetized bar of iron or steel, or a solenoid. Magnetic field generator 22 further includes a means for varying the magnetic field to be applied to magnet 12. If generator 22 includes a solenoid, the current applied to the solenoid may be varied so as to vary the strength of the solenoid's magnetic field. If generator 22 includes a bar magnet, it will also include a means for moving that magnet closer or nearer to magnet 12 so as to vary the magnetic field applied to magnet 12. One skilled in the art will appreciate that any well known method of imparting movement may be used to move the magnet, including a cam or motor driven push rod or screw. To place an axial load on construct 20, one skilled in the art will also appreciate that if magnet 12 is magnetized, field generator 22 need not also be magnetized, and need only comprise a material that is attracted by an external magnetic field.

By varying the magnetic field applied to magnet 12, the axial load on the construct may likewise be varied. It will thus be appreciated that the axial load may be varied to any extent and at any interval, or may be maintained as a constant. This is advantageous as the ideal axial load to be applied to the construct will vary with time due to the increasing density of the construct during seeding and culturing. Moreover, the ideal axial load to be applied necessarily depends from the outset on the original length and thickness of the construct, and may thus vary from treatment to treatment.

Perhaps most importantly, axial load variation is advantageous because stress is placed on construct 10 which resembles the physiological conditions typically encountered by tendons or ligaments in the human body. These culturing conditions are advantageous as they may improve the flow of nutrients to and removal of waste products from cells embedded in the construct. These conditions are also advantageous as they can be detected by living cells attached to construct 20, thus causing the cells to align and configure themselves in a manner more likely to tolerate the physiological conditions found in the human body.

FIG. 2 discloses an alternative embodiment of a system for sterilizing, seeding, culturing, storing, shipping, and testing tendon and ligament constructs. This system primarily comprises a treatment chamber 50, a pump 30, a piston 54, and a media reservoir 18 communicating with the treatment chamber.

Pump 30, media reservoir 18, fluid lines 14 and 15, ports 16 and 17, and the fluids which the system may contain are the same as those disclosed in conjunction with FIG. 1. Chamber 50 is identical to chamber 10, except chamber 50, unlike chamber 10, is sealed at one end by a piston. As with the system disclosed in FIG. 1, the fluid contained in reservoir 18 may be retrieved through fluid lines 14 or 15 into treatment chamber 50 through the action of pump 30, which controls the unidirectional or bidirectional flow and pressure of the fluid within the system. Also, as with the system disclosed in FIG. 1, construct 20 is secured to one end of treatment chamber 50. As set forth below, what differs from the embodiment disclosed in FIG. 1 is the means by which an axial load is placed on construct 20.

As shown in FIG. 2, the end of chamber 50 at which construct 20 is attached is also attached to a fixed structure 52. The opposite end of construct 20 is attached

to a piston 54. Piston 54 is configured and dimensioned, using for example an o-ring, to create a hermetically sealed chamber 50. Force is applied to piston 54 in either direction by a force generator 56. Force generator 56 comprises any well known means for providing bidirectional linear force to a piston, such as an electrically-driven rotating cam, an electromechanical or pneumatic displacement device, or an electrically or pneumatically-driven lever arm. Alternatively, force may be applied to piston 54 by varying the fluid flow, and thus the pressure, within chamber 10. It is to be understood that the force applied to piston 54 may be varied so as to provide a varying axial load on construct 20 within treatment chamber 50 during use. As with the system disclosed in FIG. 1, this varying axial load is advantageous because stress is placed on construct 20 which resembles the physiological conditions typically encountered by tendons or ligaments in the human body. This is additionally advantageous as the ideal load to be applied to the construct will vary with time due to the increasing density of the construct during seeding and culturing. Moreover, the ideal load to be applied necessarily depends from the outset on the original length and thickness of the construct, and may thus vary from treatment to treatment.

FIG. 3 discloses yet another alternative embodiment of the invention for sterilizing, seeding, culturing, storing, shipping, and testing tendon or ligament constructs. According to this alternative exemplary embodiment of the invention, the system primarily comprises a bellows 100, a pump 30, and a media reservoir 18.

Pump 30, media reservoir 18, fluid lines 14 and 15, ports 16 and 17, and the fluids which the system may contain are the same as those disclosed in conjunction with FIGS. 1 and 2. As with the systems disclosed in FIGS. 1 and 2, the fluid contained in reservoir 18 may be retrieved through

fluid lines 14 or 15 into treatment chamber 10 through the action of pump 30, which controls the unidirectional or bidirectional flow and pressure of the fluid within the system.

Also, as with the system disclosed in FIG. 2, construct 20 is secured to one end of treatment chamber 10, with that end of chamber 10 likewise attached to a fixed structure 52. What differs from the embodiments disclosed in FIGS. 1 and 2 is the means by which an axial load is placed on construct 20. In particular, the opposite end of construct 20 is attached to bellows 100, rather than a piston or magnet.

Bellows 100 may comprise a hard-sided blow molded collapsible bellows cassette. However, one skilled in the art will understand that other types of bellows which include at least one rigid surface and flexible edges may be used. Bellows 100 may also include an external pull ring 102 for easy expansion, and may further include a sealable slit along one of the collapsible side walls so as to place construct 20 within the bellows for treatment. However, it is to be understood that a sealable slit may be placed at any location on bellows 100.

Construct 20 is attached to bellows 100 by any well known means, such as sutures, staples, or c-clips, or may be sandwiched between two opposable interlocking structures. Construct 20 may also be attached to bellows 100 by those attachment means mentioned in conjunction with FIG. 1.

As shown in FIG. 3, a force may be applied to bellows 100 by force generator 56 so as to apply an axial load to construct 20. As mentioned in conjunction with FIG. 2, force generator 56 comprises any well known means for providing force in two directions, such as an electrically-driven rotating cam, an electromechanical or pneumatic displacement device, or an electrically or pneumatically-driven lever arm. Alternatively, force may be applied to bellows 100 by varying

the fluid flow, and thus pressure, within the bellows. It is also to be understood that the force applied to bellows 100 may be varied so as to provide a varying axial load on construct 20 within bellows 100 during use. Like the systems of FIGS. 1 and 2, an axial load is thus accomplished during seeding, culturing, and testing which closely resembles the physiological conditions found in the human body.

FIG. 4 discloses yet another alternative embodiment of the invention for sterilizing, seeding, culturing, storing, shipping, and testing tendon and ligament constructs. According to this alternative exemplary embodiment of the invention, the system primarily comprises a chamber 150, a pump 30, and a media reservoir 18. Pump 30, media reservoir 18, fluid lines 14 and 15, ports 16 and 17, and the fluids which the system may contain are the same as those disclosed in conjunction with FIGS. 1-3.

As shown in FIG. 4, chamber 150 comprises rigid upper and lower frame members 152 and 153 connected by a flexible diaphragm 154. Construct 20 is attached to opposing sides of flexible diaphragm 154 by any well known means, including those mentioned in conjunction with FIGS. 1 and 3.

In the exemplary embodiment of the invention disclosed in FIG. 4, pump 30 controls both the flow of the fluid within treatment chamber 150 and the pressure within that treatment chamber. Pump 30 may be any pump capable of providing unidirectional or bidirectional pulsatile or continuous pressure fluid flow in the system. Examples of such pumps include but are not limited to low flow pumps, invasive pumps such as a lobe-type pump, or non-invasive pumps such as a peristaltic pump. Thus, illustratively, when pump 30 is operating so as to force fluid from reservoir 18 to fluid line 14, and subsequently from fluid line 14 into chamber 150, fluid is forced from port 16 through chamber 150 to port 17. However, if pump 30 is operated so as to provide fluid

flow in the opposite direction, fluid is then forced in an opposite direction from port 17 through chamber 150 to port 16.

It is to be understood that the pressure from pump 30 may be varied during use so as to provide varying pressure within treatment chamber 150. By varying the pressure from pump 30, flexible diaphragm 154 may be expanded and contracted. By expanding and contracting flexible diaphragm 154, a varying axial load is placed on construct 20, attached as mentioned to opposing sides of diaphragm 154. This varying axial load is advantageous because an axial stress is placed on construct 20 which resembles the physiological conditions typically encountered by tendons or ligaments in the human body. This is additionally advantageous as the ideal load to be applied to the construct will vary with time due to the increasing density of the construct during seeding and culturing. Moreover, the ideal load to be applied necessarily depends from the outset on the original length and thickness of the construct, and may thus vary from treatment to treatment.

In an alternative embodiment of the system disclosed in FIG. 4, only one port to chamber 150 is provided. As in the two-port embodiment, the one port may be used to circulate fluids into and out of treatment chamber 150, and to pressurize and depressurize chamber 150. One skilled in the art will understand that if only one port is used, pressure can be applied using, for example, a pump, piston or pressurized air.

It is to be understood that any ports of treatment chamber 10 (in FIG. 1), chamber 50 (in FIG. 2), bellows 100 (in FIG. 3), and chamber 150 (in FIG. 4) (hereinafter collectively referred to as the "treatment devices") may be sealed in a known manner (e.g., luer locks, o-ring based connectors, or threaded plugs) so as to create a sealed

treatment device free from contamination. The sealed treatment devices may be used to sterilize, store, and ship tendon and ligament constructs or other protheses. In particular, prior to placing a sealed treatment device into the systems of FIGS. 1 - 4, a construct 20 which is secured within the treatment device may be sterilized by some chemical means such as ethylene oxide or peracetic acid, radiation means such as an electron beam or gamma rays, or by steam sterilization. Sealed treatment devices, containing the sterilized tendon or ligament support material, may then be placed back into the systems of FIGS. 1 - 4 for seeding and culturing and unsealed without contaminating the system or the tissue construct. Alternatively, the system may be aseptically assembled after sterilization if it is necessary or desirable to use different means to sterilize the treatment devices and the tissue construct.

Seeding and culturing of the constructs in the systems disclosed in FIGS. 1 - 4 is generally accomplished by known techniques, with the added benefits and advantages gained from the stress placed upon the construct during seeding or growth steps. Examples of suitable seeding and culturing methods for the growth of three-dimensional tissue cultures are disclosed in U.S. Application No. 08/463,566, entitled "Three-Dimensional Cartilage Cultures" and filed on June 5, 1995, and U.S. Patent No. 5,266,480, both of which are incorporated herein by reference. The techniques described in this application and U.S. Patent for establishing a three-dimensional construct, inoculating the construct with the desired cells, and maintaining the culture may be readily adapted by a person of ordinary skill in the art for use with the present invention.

Once construct 20 has reached the desired level of cell density, a preservative may then be pumped into the treatment device. Once the device is filled with the preservative, any

ports located on the device may be closed, again creating a sealed device which may then be used to store and/or ship the cultured and preserved construct. Preferably, the preservative is a cryo-preservative so that the construct may be frozen in the treatment device. In this manner, the sealed treatment devices may be used to sterilize, culture, store, and ship tendon and ligament constructs or other protheses.

Various embodiments of the invention have been described. The descriptions are intended to be illustrative, not limitative. Thus, it will be apparent to those skilled in the art that modifications may be made to the invention as described without departing from the scope of the claims set out below. For example, it will be recognized that the exemplary embodiments disclosed in conjunction with FIGS. 1-4 need not have both an inlet and an outlet port to apply an axial stress to the construct during culturing, but instead may have a plurality of ports, one port, or no ports. Likewise, if only a constant axial load on the tissue construct is desired, magnet 12 in FIG. 1 may, for example, be replaced with a non-magnetized dead weight. Similarly, an axial load can be placed on the constructs shown in FIGS. 2, 3, and 4 by varying the pressure external to the treatment chamber through, for example, the creation of a vacuum around the exterior of the chamber. This is advantageous as fluid access to the treatment chamber is not required.

We claim:

1. An apparatus for tissue growth, comprising:

a substrate designed to facilitate three-dimensional tissue growth on said substrate, said substrate comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

a housing defining a tissue growth chamber;

a support structure located within said chamber configured and dimensioned to support said substrate; and

means for controlling media flow characteristics around said substrate mounted on said support structure within said housing so as to simulate the physiological conditions to be encountered by tissue grown on said substrate once implanted.

2. The apparatus of claim 1, wherein said housing includes a first port and a second port for flow of media therethrough.

3. The apparatus of claim 2, wherein said controlling means controls media flow characteristics as said media flows from the first port through said chamber to the second port.

4. The apparatus of claim 3, wherein said controlling means includes a pump in fluid communication with said first and second ports.

5. The apparatus of claim 1, wherein said substrate is configured and dimensioned as a heart valve.

6. The apparatus of claim 1, wherein said substrate is configured and dimensioned as a vascular graft.

7. The apparatus of claim 1, wherein said substrate is configured and dimensioned as a cartilage graft.

8. An apparatus for tissue growth, comprising:

a substrate designed to facilitate three-dimensional tissue growth on said substrate, said substrate comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells;

a housing defining a tissue growth chamber;

a support structure located within said chamber configured and dimensioned to support said substrate; and

means for moving said support structure between a first position and a second position, wherein movement of said support structure between said positions creates varying stresses in the substrate supported thereby, said varying stresses simulating the physiological conditions to be encountered by tissue grown on said substrate once implanted.

9. The apparatus of claim 8, wherein said housing includes a first port and a second port for flow of media therethrough.

10. The apparatus of claim 8, wherein said moving means includes a piston.

11. The apparatus of claim 9, wherein said moving means includes a pump in fluid communication with said first and second ports.

12. The apparatus of claim 8, wherein the support structure comprises an expandable member that is adapted to receive the substrate thereover.

13. The apparatus of claim 8, wherein the support structure comprises a bellows.

14. The apparatus of claim 8, wherein said substrate is configured and dimensioned as a vascular graft.

15. An apparatus for tissue growth, comprising:
a housing defining a seeding and culturing chamber;
a support structure located within said chamber configured and dimensioned to support a substrate; and
means for imparting an axial stress to the substrate mounted on said support structure within said chamber.

16. The apparatus of claim 15, wherein said housing includes a first port and a second port for flow of fluid media therethrough.

17. The apparatus of claim 15, wherein said imparting means comprises a means for applying an axial magnetic load to the substrate.

18. The apparatus of claim 15, wherein said imparting means comprises a means for applying an axial mechanical load to the substrate.

19. The apparatus of claim 18, wherein said applying means comprises a piston.

20. The apparatus of claim 18, wherein said applying means comprises a bellows.

21. The apparatus of claim 18, wherein said imparting means comprises a flexible diaphragm.

22. The apparatus of claim 15, wherein said support structure comprises a plurality of sutures.

23. The apparatus of claim 16, further comprising a pump fluidly connected to said first and second ports for providing varying fluid flow and pressure within said chamber.

24. The apparatus of claim 16, wherein the first and second ports of said housing may be sealed for enclosing, sterilizing, storing, and shipping the substrate.

25. The apparatus of claim 15, further comprising a substrate designed to facilitate three-dimensional tissue growth on said substrate, said substrate comprising a biocompatible, non-living three-dimensional framework having interstitial spaces bridgeable by cells.

26. A method for seeding and culturing a substrate, comprising:

exposing the substrate to a fluid media for seeding and culturing; and

imparting an axial stress to the substrate during said seeding and culturing to encourage a desired alignment of cells on the substrate.

27. The method of claim 26, wherein said step of imparting axial stress comprises:

placing said substrate on a support structure; and

moving said substrate between a first position and a second position so that axial stress is imparted to the substrate.

28. The method of claim 27, wherein the length of said substrate is varied by said step of moving said prosthesis.

29. A method for seeding and culturing a substrate, comprising:

exposing the substrate to a fluid media for seeding and culturing; and

imparting stresses to the substrate during said seeding and culturing to simulate the physiological conditions to be encountered by the tissue grown on said substrate once implanted, thereby encouraging a desired alignment of cells on the substrate.

30. The method of claim 29, wherein said step of imparting stresses comprises:

placing said substrate on a support structure; and

moving said substrate between a first position and a second position so that stress is imparted to the prosthesis.

31. The method of claim 29, wherein said step of imparting stresses comprises:

placing said substrate on a support structure; and

controlling media flow characteristics around said substrate mounted on said support structure.

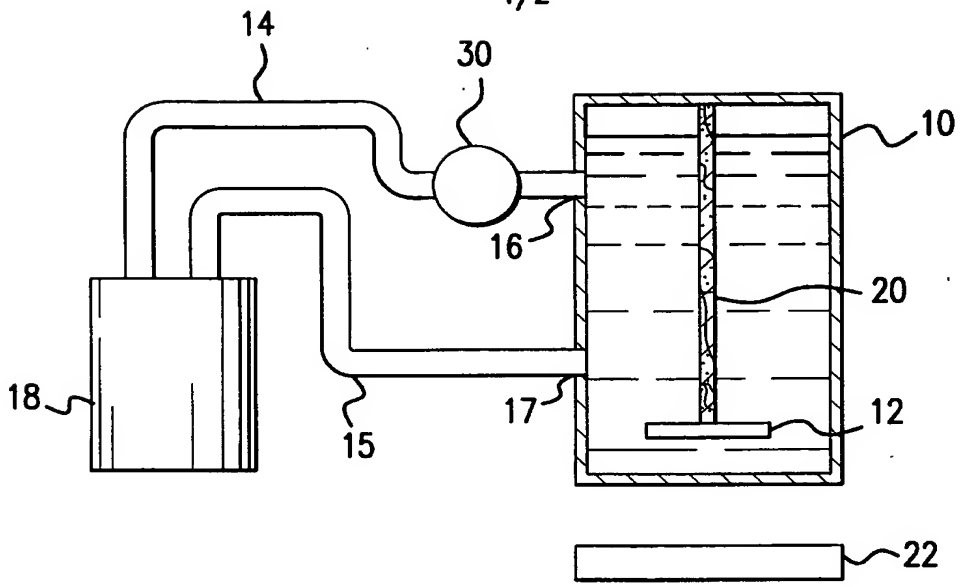


FIG.1

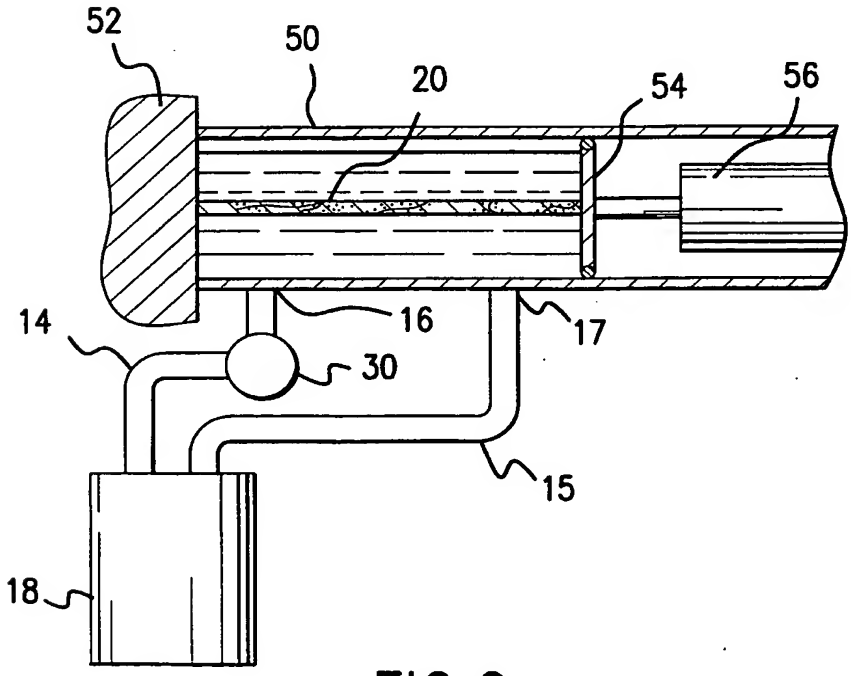


FIG.2

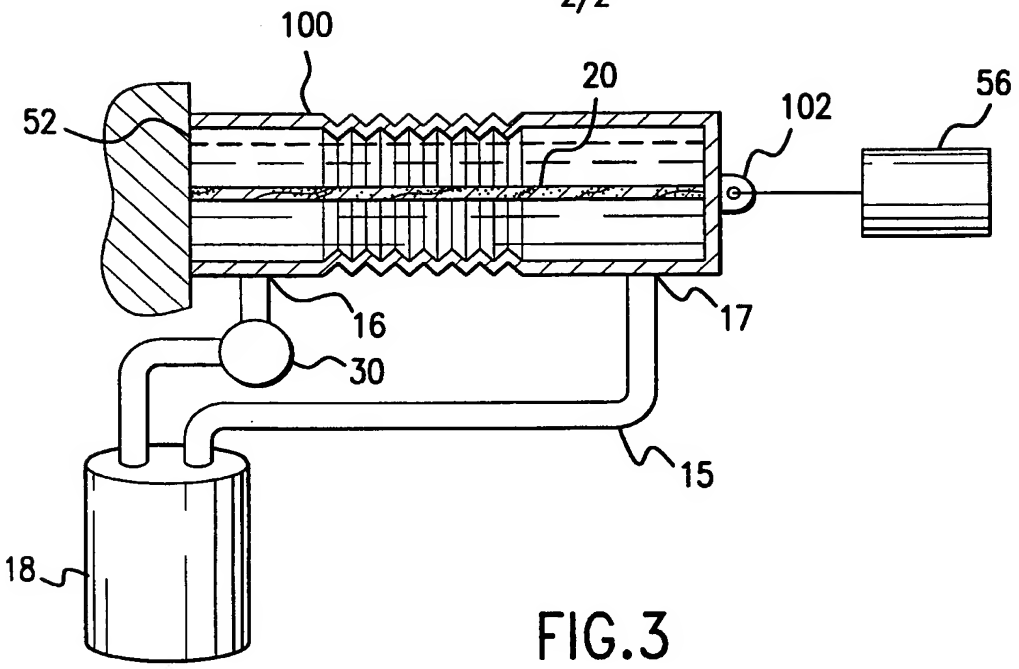


FIG.3

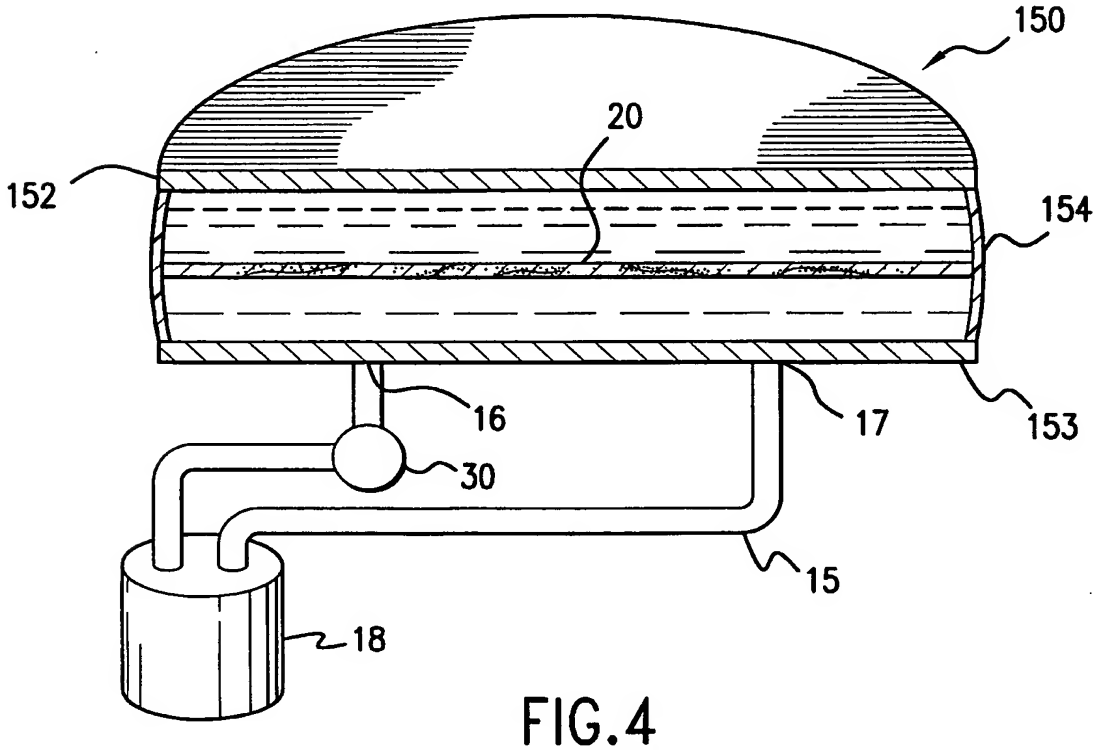


FIG.4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 98/27845

A. CLASSIFICATION OF SUBJECT MATTER

C 12 M 3/00, C 12 N 5/00

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C 12 M, C 12 N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5266480 A (NAUGHTON et al.) 30 November 1993 (30.11.93), claims 1,11,21. --	1,8, 15,25, 26,29
A	WO 97/38777 A1 (ADVANCED TISSUE SCIENCES, INC.) 23 October 1997 (23.10.97), claims 1-5,23. ----	1-4,8, 9,11, 15,26, 29

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 1999	03.06.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	WOLF e.h.

Form PCT/ISA/210 (second sheet) (July 1992)